

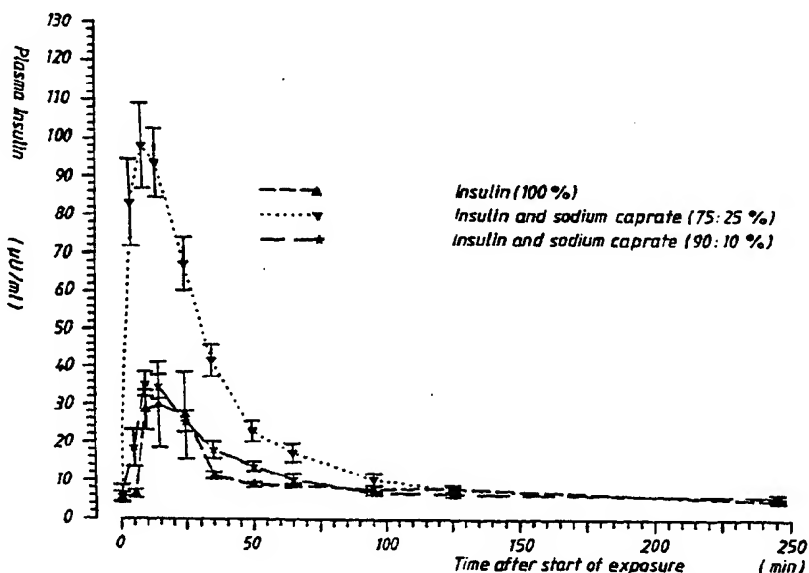
PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|--|--|---|---|
| (51) International Patent Classification ⁶ : A61K 9/72, 9/14, 37/26, 47/12 | | A1 | (11) International Publication Number: WO 95/00128 |
| | | | (43) International Publication Date: 5 January 1995 (05.01.95) |
| (21) International Application Number: PCT/SE94/00634 | | (81) Designated States: AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). | |
| (22) International Filing Date: 23 June 1994 (23.06.94) | | | |
| (30) Priority Data: 9302198-8 24 June 1993 (24.06.93) SE 9400371-2 4 February 1994 (04.02.94) SE | | | |
| (71) Applicant: ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE). | | Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> | |
| (72) Inventors: BÄCKSTRÖM, Kjell, Göran, Erik; Notariegränden 4, S-226 47 Lund (SE). DAHLBÄCK, Carl, Magnus, Olof; Sköldgränden 10, S-224 75 Lund (SE). EDMAN, Peter; Kamrersvägen 18, S-237 34 Bjärred (SE). JOHANSSON, Ann, Charlotte, Birgit; Arkeologvägen 65, S-226 54 Lund (SE). | | | |

(54) Title: COMPOSITIONS FOR INHALATION



(57) Abstract

Pharmaceutical compositions containing a mixture of a pharmaceutically active polypeptide and an enhancer compound which enhances the systemic absorption of the polypeptide in the lung of a patient, the mixture being in the form of a dry powder, in which at least 50 % of the total mass of polypeptide and enhancer consists of primary particles having a diameter less than or equal to about 10 microns, the primary particles optionally being formed into agglomerates; and methods of delivering such compositions by inhalation.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | |
|----|--------------------------|----|---------------------------------------|----|--------------------------|
| AT | Austria | GB | United Kingdom | MR | Mauritania |
| AU | Australia | GE | Georgia | MW | Malawi |
| BB | Barbados | GN | Guinea | NE | Niger |
| BE | Belgium | GR | Greece | NL | Netherlands |
| BF | Burkina Faso | HU | Hungary | NO | Norway |
| BG | Bulgaria | IE | Ireland | NZ | New Zealand |
| BJ | Benin | IT | Italy | PL | Poland |
| BR | Brazil | JP | Japan | PT | Portugal |
| BY | Belarus | KE | Kenya | RO | Romania |
| CA | Canada | KG | Kyrgyzstan | RU | Russian Federation |
| CF | Central African Republic | KP | Democratic People's Republic of Korea | SD | Sudan |
| CG | Congo | KR | Republic of Korea | SE | Sweden |
| CH | Switzerland | KZ | Kazakhstan | SI | Slovenia |
| CI | Côte d'Ivoire | LI | Liechtenstein | SK | Slovakia |
| CM | Cameroon | LK | Sri Lanka | SN | Senegal |
| CN | China | LU | Luxembourg | TD | Chad |
| CS | Czechoslovakia | LV | Latvia | TG | Togo |
| CZ | Czech Republic | MC | Monaco | TJ | Tajikistan |
| DE | Germany | MD | Republic of Moldova | TT | Trinidad and Tobago |
| DK | Denmark | MG | Madagascar | UA | Ukraine |
| ES | Spain | ML | Mali | US | United States of America |
| FI | Finland | MN | Mongolia | UZ | Uzbekistan |
| FR | France | | | VN | Viet Nam |
| GA | Gabon | | | | |

COMPOSITIONS FOR INHALATION

5 This invention relates to methods and compositions for delivery of medically useful peptides and proteins.

Background of the Invention

Although the advent of recombinant DNA technology has resulted in a rapidly expanding list of peptide-based drugs, a major drawback of peptide-based therapy has acutely hampered realization of the full potential of this field: in
10 general, peptide-based drugs cannot be orally administered in effective doses, since they are rapidly degraded by enzymes in the gastrointestinal tract before they can reach the bloodstream. Unless the polypeptide of interest can be altered to make it relatively resistant to such enzymes, the only practical method of delivering the
15 drug is likely to be a parenteral route, such as by intravenous, intramuscular, or subcutaneous injection. Administration by other parenteral routes (e.g., by absorption across nasal, buccal or rectal membranes, or via the lung) has met with limited success.

Summary of the Invention

20 It has been found that when a peptide or protein (hereinafter collectively referred to as polypeptides) is combined with an appropriate absorption enhancer and is introduced into the lung in the form of a powder of appropriate particle size, it readily enters the pulmonary circulation by absorption through the
25 layer of epithelial cells in the lower respiratory tract. This is conveniently accomplished by inhalation of the powder from an inhaler device which dispenses the correct dose of powdered polypeptide/enhancer in a particle size which maximizes deposition in the lower respiratory tract, as opposed to the mouth and throat. (For ease of reference, the polypeptide and enhancer are hereinafter
30 collectively referred to as the "active compounds"). To accomplish this preferential

delivery into the lung, as much as possible of the active compounds should consist of particles having a diameter less than approximately 10 μm (e.g., between 0.01-10 μm , and ideally between 1-6 μm). In preferred embodiments, at least 50% (preferably at least 60%, more preferably at least 70%, still more preferably at least 80%, and most preferably at least 90%) of the total mass of active compounds which exits the inhaler device consists of particles within the desired diameter range.

The invention thus includes a pharmaceutical composition containing a mixture of active compounds (A) a pharmaceutically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory system (preferably the lungs) of a patient, the mixture being in the form of a dry powder suitable for inhalation, in which at least 50% of the total mass of active compounds (A) and (B) consists of primary particles having a diameter less than or equal to about 10 microns. The primary particles may be packaged as such, or may optionally be formed into agglomerates, which then are substantially deagglomerated prior to entry into the respiratory tract of the patient. The composition may of course contain other ingredients as needed, including other pharmaceutically active agents, other enhancers, and pharmacologically acceptable excipients such as diluents or carriers. Therefore, the therapeutic preparation of the present invention may contain only the said active compounds or it may contain other substances, such as a pharmaceutically acceptable carrier. This carrier may largely consist of particles having a diameter of less than about 10 microns so that at least 50 % of the resultant powder as a whole consists of optionally agglomerated primary particles having a diameter of less than about 10 microns; alternatively the carrier may largely consist of much bigger particles ("coarse particles"), so that an "ordered mixture" may be formed between the active compounds and the said carrier. In an ordered mixture, alternatively known as an interactive or adhesive mixture, fine drug particles (in this invention, the active compounds) are fairly evenly distributed over the surface of coarse excipient particles (in this invention, the pharmaceutically acceptable

carrier). Preferably in such case the active compounds are not in the form of agglomerates prior to formation of the ordered mixture. The coarse particles may have a diameter of over 20 microns, such as over 60 microns. Above these lower limits, the diameter of the coarse particles is not of critical importance so various
5 coarse particle sizes may be used, if desired according to the practical requirements of the particular formulation. There is no requirement for the coarse particles in the ordered mixture to be of the same size, but the coarse particles may advantageously be of similar size within the ordered mixture. Preferably, the coarse particles have a diameter of 60 - 800 microns.

10

The polypeptide may be any medically or diagnostically useful peptide or protein of small to medium size, i.e. up to about 40 kD molecular weight (MW), for which systemic delivery is desired. The mechanisms of
15 improved polypeptide absorption according to the present invention are generally applicable and should apply to all such polypeptides, although the degree to which their absorption is improved may vary according to the MW and the physico-chemical properties of the polypeptide, and the particular enhancer used. It is expected that polypeptides having a molecular weight of up to 30 kD will be most
20 useful in the present invention, such as polypeptides having a molecular weight of up to 25 kD or up to 20 kD, and especially up to 15 kD or up to 10kD. Any desired polypeptide may be easily tested for use in the present invention with a particular enhancer, by *in vivo* or *in vitro* assays, as described herein.

25

The enhancer compound used in the compositions of the present invention can be any compound which enhances the absorption of the polypeptide through the epithelium of the lower respiratory tract, and into the systemic circulation. By "enhances absorption" is meant that the amount of polypeptide

30

absorbed into the systemic circulation in the presence of enhancer is higher than in the absence of enhancer. Preferably the amount of polypeptide absorbed is significantly higher ($p < 0.05$) in the presence of enhancer. The suitability of any potential enhancer for use in the present invention may be easily assessed, by means of *in vivo* or *in vitro* assays, as described herein.

The amount of polypeptide absorbed according to the present invention is preferably at least 150% of the amount absorbed in the absence of enhancer. In preferred embodiments, absorption of polypeptide is at least doubled, more preferably tripled, and most preferably quadrupled in the presence of the enhancer, compared to in its absence.

The enhancer is preferably a surfactant such as a salt of a fatty acid, a bile salt, a bile salt derivative, an alkyl glycoside, a cyclodextrin, or a phospholipid. The enhancer may be, for example, a sodium, potassium, or organic amine salt of the fatty acid, and the fatty acid is preferably capric acid or another fatty acid of 10-14 carbon atoms. The preferred enhancer is sodium caprate. The ratio of polypeptide to enhancer will preferably vary from about 9:1 to about 1:1. Although proportions of enhancer greater than 1:1 would presumably enhance uptake as well as or better than lower proportions, it is believed that the amount of enhancer used should be no higher than necessary to achieve the desired level of enhancement, since excess enhancer may trigger unwanted side effects, such as local irritation.

Also within the invention is a method of administering systemically a pharmaceutically active polypeptide, by causing a patient to inhale the pharmaceutical composition of the invention, wherein at least 50% of the total mass of the active compounds at the point of entry to the respiratory tract of the patient consists of particles having a diameter less than or equal to about 10 microns. This is preferably accomplished by the use of an inhaler device from which the patient inhales the powder. Where the powdered composition is in the form of agglomerates of primary particles, the device is preferably configured to

induce substantial deagglomeration of the agglomerates upon inhalation of the powder from the device by the patient, so that the majority of the agglomerates break down into particles having a diameter less than or equal to about 10 microns, prior to entry of the powder into the respiratory system of the patient. This
5 deagglomeration would occur inside the device, and is typically induced by the air turbulence created in the device by the force of inhalation. Agglomerates are in general preferably not formed in the ordered mixture. In the case of an ordered mixture, the active compounds should be released from the large particles preferably upon inhalation, either by mechanical means in the inhaler device or
10 simply by the action of inhalation, or by other means, the active compounds then being deposited in the lower respiratory tract and the carrier particles in the mouth.

The inhaler device is preferably a single dose dry powder inhaler, but may alternatively be a multi dose dry powder inhaler.

15

The invention also includes processes for the manufacture of a pharmaceutical composition suitable for administration by inhalation. In one such process, a solution is first provided in which are dissolved (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the systemic
20 absorption of the polypeptide in the lower respiratory tract of a patient. The solvent is then removed from the solution to yield a dry solid containing the polypeptide and the enhancer, and the dry solid is pulverized to produce a powder. A second such process involves dry mixing (a) a pharmaceutically active polypeptide and (b) an enhancer compound, and micronizing the obtained mixture.
25 Yet a third suitable process includes the steps of providing a first micronized preparation containing a polypeptide and a second micronized preparation containing an enhancer compound, and mixing the two micronized preparations together. When a carrier is to be included other than when an ordered mixture is desired, this may be added to the solution, or to the dry-mixture of the
30 pharmaceutically active polypeptide prior to micronization, or micronised carrier

may be dry mixed with the other micronised components. In producing an ordered mixture, micronised polypeptide and enhancer are mixed with a suitable carrier.

Brief Description of the Drawings

5 Fig. 1 is a graph illustrating the effects of different concentrations of sodium caprate enhancer on the transport of a marker compound (mannitol) through a monolayer of cultured epithelial cells.

Fig. 2 is a graph illustrating the effects of different concentrations of sodium caprate enhancer on the transport of a marker compound (mannitol)
10 through a monolayer of cultured epithelial cells, in the presence of a polypeptide (sodium caprate:polypeptide 1:3 by weight).

Fig. 3 is a graph of plasma polypeptide concentration as a function of time after inhalation of the polypeptide alone, the polypeptide with sodium caprate in a ratio of 90:10, and the polypeptide with sodium caprate in a ratio of 75:25.
15

Detailed Description

Some of the preferred embodiments of the invention are generally described below.

20

The Polypeptide

The polypeptide is preferably a peptide hormone other than insulin, such as vasopressin, vasopressin analogues, desmopressin, glucagon, corticotropin (ACTH), gonadotrophin (luteinizing hormone, or LHRH), calcitonin, C-peptide of
25 insulin, parathyroid hormone (PTH), human growth hormone (hGH), growth hormone (HG), growth hormone releasing hormone (GHRH), oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs, gonadotropin agonist analogs (GnRHa), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRHrh), follicle stimulating hormone (FSH), and prolactin.

30

Other possible polypeptides include growth factors, interleukins,

polypeptide vaccines, enzymes, endorphins, glycoproteins, lipoproteins, and polypeptides involved in the blood coagulation cascade, that exert their pharmacological effect systemically. It is expected that most if not all polypeptides of small to medium size, relatively high water solubility, and an isoelectric point between approximately pH 3 and pH 8 can be effectively delivered by the methods of the invention.

The Enhancer

The use of an absorption enhancer is of critical importance, as the polypeptide alone is poorly absorbed through the lung. The enhancer used can be any of a number of compounds which act to enhance absorption through the layer of epithelial cells lining the lower respiratory tract, and into the adjacent pulmonary vasculature. The enhancer can accomplish this by any of several possible mechanisms:

(1) Enhancement of the paracellular permeability of a polypeptide by inducing structural changes in the tight junctions between the epithelial cells.

(2) Enhancement of the transcellular permeability of a polypeptide by interacting with or extracting protein or lipid constituents of the membrane, and thereby perturbing the membrane's integrity.

(3) Interaction between enhancer and polypeptide which increases the solubility of the polypeptide in aqueous solution. This may occur by preventing formation of insulin aggregates (dimers, trimers, hexamers), or by solubilizing polypeptide molecules in enhancer micelles.

(4) Decreasing the viscosity of, or dissolving, the mucus barrier lining the alveoli and passages of the lung, thereby exposing the epithelial surface for direct absorption of the polypeptide.

Enhancers may function by only a single mechanism set forth above, or by two or more. An enhancer acting by several mechanisms is more likely to promote efficient absorption of a polypeptide than one which employs only one or two.

For example, surfactants are a class of enhancers which are believed to act by all four mechanisms listed above. Surfactants are amphiphilic molecules having both a lipophilic and a hydrophilic moiety, with varying balance between these two characteristics. If the molecule is very lipophilic, the low solubility of the substance in water may limit its usefulness. If the hydrophilic part overwhelmingly dominates, however, the surface active properties of the molecule may be minimal. To be effective, therefore, the surfactant must strike an appropriate balance between sufficient solubility and sufficient surface activity.

Another surfactant property that may be of importance is the net charge of the surfactant at the pH value in the lung (approximately 7.4). At pH 7.4, some polypeptides have a negative net charge. This will result in an electrostatic repulsion between molecules, which will in turn prevent aggregation and thereby increase the solubility. If the surfactant also is negatively charged, it can interact with the polypeptide by, for example, hydrophobic interactions, and additional repulsion among the polypeptide molecules will occur. In such case an anionic surfactant will possess the additional advantage (compared to those having neutral or net positive charge at physiological pH) of enhancing absorption by helping stabilize the polypeptide in the monomeric state.

A number of different compounds potentially useful as enhancers in the methods of the invention were tested in rats, as described in Example 2 below. Other substances with known absorption-enhancing properties, or with physical characteristics which make them likely candidates for use in the method of the invention, can be readily tested by one of ordinary skill in that *in vivo* assay, or alternatively in the *in vitro* assay described in Example 1.

It is possible that a combination of two or more enhancer substances also gives satisfactory results. The use of such a combination in the method of the invention is considered to be within the invention.

An enhancer useful in the methods of the invention will combine effective enhancement of polypeptide absorption with (1) lack of toxicity in the concentrations used and (2) good powder properties, i.e., lack of a sticky or waxy

consistency in the solid state. Toxicity of a given substance can be tested by standard means, such as by the MTT assay, for example as described in Int. J. Pharm., 65 (1990), 249-259. The powder properties of a given substance may be ascertained from published data on the substance, or empirically.

5 One very promising type of enhancer is the salt of a fatty acid. It has been found that the sodium salt of saturated fatty acids of carbon chain length 10 (i.e., sodium caprate), 12 (sodium laurate) and 14 (sodium myristate) perform well in the method of the invention. The potassium and lysine salts of capric acid have also been found to be effective in the method of the invention. If the carbon chain
10 length is shorter than about 10, the surface activity of the surfactant may be too low, and if the chain length is longer than about 14, decreased solubility of the fatty acid salt in water limits its usefulness.

 Most preferably in the present invention the substance which enhances the absorption of polypeptide in the lower respiratory tract is sodium caprate.

15 Different counterions may change the solubility of the saturated fatty acid salt in water, such that an enhancer having a carbon length other than 10 - 14 would prove even more advantageous than the enhancers specifically mentioned hereinabove. Salts of unsaturated fatty acids may also be useful in the present invention since they are more water soluble than salts of saturated fatty acids, and
20 can therefore have a longer chain length than the latter and still maintain the solubility necessary for a successful enhancer of polypeptide absorption.

 All of the bile salts and bile salt derivatives tested (sodium salts of ursodeoxycholate, taurocholate, glycocholate, and taurodihydrofusidate) effectively enhance polypeptide absorption in the lung.

25 Phospholipids were also tested as enhancers. It was found that a single-chain phospholipid (lysophosphatidylcholine) was an effective enhancer, while two double-chain phospholipids (dioctanoylphosphatidylcholine and didecanoylphosphatidylcholine) were not. This may be explained by the fact that the double-chain phospholipids are much less soluble in water than their single-
30 chain counterparts; however, it is reasonable to expect that double-chain

phospholipids of shorter chain length, having greater water-solubility than their longer chain counterparts, will be of use as enhancers in the present invention so that both single- and double-chain phospholipids may be used.

One glycoside, octylglucopyranoside, was tested as an enhancer in the present invention and was found to have some absorption enhancing properties. Other alkyl glycosides, such as thioglucopyranosides and maltopyranosides would also be expected to exhibit absorption enhancing properties in the methods of the present invention.

The cyclodextrins and derivatives thereof effectively enhance nasal absorption, and may function similarly in the lung. Dimethyl- β -cyclodextrin has been tested and was found to have an absorption enhancing effect.

Other potentially useful surfactants are sodium salicylate, sodium 5-methoxysalicylate, and the naturally occurring surfactants such as salts of glycyrrhizine acid, saponin glycosides and acyl carnitines.

For ionic enhancers (e.g., the anionic surfactants described above), the nature of the counterion may be important. The particular counterion selected may influence the powder properties, solubility, stability, hygroscopicity, and local/systemic toxicity of the enhancer or of any formulation containing the enhancer. It may also affect the stability and/or solubility of the polypeptide with which it is combined. In general, it is expected that monovalent metallic cations such as sodium, potassium, lithium, rubidium, and cesium will be useful as counterions for anionic enhancers. Ammonia and organic amines form another class of cations that is expected to be appropriate for use with anionic enhancers having a carboxylic acid moiety. Examples of such organic amines include ethanolamine, diethanolamine, triethanolamine, 2-amino-2-methylethylamine, betaines, ethylenediamine, N,N-dibenzylethylenetetraamine, arginine, hexamethylenetetraamine, histidine, N-methylpiperidine, lysine, piperazine, spermidine, spermine and tris(hydroxymethyl)aminomethane.

Since effective enhancement of polypeptide absorption in the lung was observed for a number of the enhancers tested, it is expected that many more will

be found which also function in this manner. Starch microspheres effectively enhance the bioavailability of polypeptide delivered via the nasal membranes and were tested as an enhancer in the methods of the invention. Although they proved to be of little use for delivery via the pulmonary route in the animal model utilized
5 herein, it is thought that this was mainly due to technical difficulties which, if overcome, may lead to successful delivery via the pulmonary route.

Chelators are a class of enhancers that are believed to act by binding calcium ions. Since calcium ions help maintain the dimensions of the space between cells and additionally reduce the solubility of a polypeptide, binding of
10 these ions would in theory both increase the solubility of polypeptides, and increase the paracellular permeability of polypeptides. Although one chelator tested, the sodium salt of ethylenediaminetetraacetic acid (EDTA), was found to be ineffective in enhancing absorption of insulin in the rat model tested, other calcium ion-binding chelating agents may prove to be more useful.

15

Proportions of polypeptide and enhancer

The relative proportions of polypeptide and enhancer may be varied as desired. Sufficient enhancer must be present to permit efficient absorption of the inhaled polypeptide; however, the amount of enhancer should be kept as low as
20 possible in order to minimize the risk of adverse effects caused by the enhancer. Although each particular polypeptide/enhancer combination must be tested to determine the optimal proportions, it is expected that to achieve acceptable absorption of the polypeptide, more than 10% of the polypeptide/enhancer mixture must be enhancer; for most types of enhancers, the proportion of enhancer should
25 be more than 15% or more than 20% and will preferably be between 25% and 50%. The preferred ratio for each polypeptide/enhancer (or polypeptide/enhancer/diluent) combination can be readily determined by one of ordinary skill in the art of pharmacology by standard methods, based on such criteria as efficient, consistent delivery of the optimal dosage, minimization of side
30 effects, and acceptable rate of absorption.

No further ingredients are needed for the action of the preparation, but may be included if desired. For example, the amount of powder which constitutes a single dose of a given polypeptide/surfactant combination could be increased (e.g., for use in an inhaler apparatus which by design requires a large powder volume per dose) by diluting the powder with pharmaceutically acceptable diluents. Other additives may be included to facilitate processing or to improve the powder properties or stability of the preparation. A flavouring agent could be added so that the proportion of the powder which is inevitably deposited in the mouth and throat would serve to give the patient positive feedback that a dose had been delivered from the inhaler device. Any such additive should have the following properties: (a) it is stable and does not disadvantageously affect the stability of the polypeptide and enhancer; (b) it does not disadvantageously interfere with absorption of the polypeptide; (c) it has good powder properties, as that term is understood in the pharmaceutical arts; (d) it is not hygroscopic; and (e) it has no adverse effects in the airways in the concentrations used. Useful types of such additives include mono-, di-, and polysaccharides, sugar alcohols, and other polyols: for example, lactose, glucose, raffinose, melezitose, lactitol, maltitol, trehalose, sucrose, mannitol, and starch. As reducing sugars such as lactose and glucose have a tendency to form complexes with proteins, non-reducing sugars such as raffinose, melezitose, lactitol, maltitol, trehalose, sucrose, mannitol and starch may be preferred additives for use in the present invention. Such additives may constitute anywhere from 0% (i.e., no additive) to nearly 100% of the total preparation.

In a preferred embodiment, this invention provides a therapeutic preparation of a pharmaceutically active polypeptide and a substance which enhances the absorption of said polypeptide in the lower respiratory tract, which preparation is in the form of a dry powder preparation suitable for inhalation of which at least 50% by mass consists of (a) particles having a diameter of less than about 10 microns or (b) agglomerates of said particles; in another preferred

embodiment, the invention provides a therapeutic preparation comprising a pharmaceutically active polypeptide, a substance which enhances the absorption of polypeptide in the lower respiratory tract, and a pharmaceutically acceptable carrier, which preparation is in the form of a dry powder suitable for inhalation of which at least 50% by mass consists of (a) particles having a diameter of less than about 10 microns, or (b) agglomerates of said particles; and in a further preferred embodiment this invention provides a therapeutic preparation comprising active compounds (A) a pharmaceutically active polypeptide and (B) a substance which enhances the absorption of said polypeptide in the lower respiratory tract, wherein at least 50 % of the total mass of active compounds (A) and (B) consists of particles having a diameter of less than about 10 microns, and a pharmaceutically acceptable carrier, which preparation is in the form of a dry powder preparation suitable for inhalation in which an ordered mixture may be formed between the active compounds and the pharmaceutically acceptable carrier.

The described powder preparation could be manufactured in several ways, using conventional techniques. In many cases, the purified polypeptide can be obtained from commercial sources. Alternatively, the polypeptide of interest can be purified from a naturally occurring source using standard biochemical techniques, or can be obtained by expression of prokaryotic or eukaryotic cells genetically engineered to contain a nucleotide sequence which encodes the polypeptide and has appropriate expression control sequences linked thereto (including a transgenic animal engineered to manufacture the desired peptide or protein, for example in its milk). Such methods are standard in the art (e.g., see Sambrook et al., Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989). Peptides (i.e., polypeptides having 30 or fewer amino acid residues) can be readily synthesized by known chemical means.

Absorption enhancers as described above are also generally available

from commercial sources, or can be manufactured using published methods. For ionic enhancers, the counterion associated with the enhancer can be replaced with another, if desired, using standard ion exchange techniques.

5 In manufacturing of the described powder preparation it will in general be necessary to micronize the powder in a suitable mill, e.g. a jet mill, at some point in the process, in order to produce primary particles in a size range appropriate for maximal deposition in the lower respiratory tract (i.e., under 10 μm). For example, one can dry mix polypeptide and enhancer powders, and
10 then micronize the substances together; alternatively, the substances can be micronized separately, and then mixed. Where the compounds to be mixed have different physical properties such as hardness and brittleness, resistance to micronisation varies and they may require different pressures to be broken down to suitable particle sizes. When micronised together, therefore, the obtained particle
15 size of one of the components may be unsatisfactory. In such case it would be advantageous to micronise the different components separately and then mix them.

 It is also possible first to dissolve the components in a suitable solvent, e.g. water, to obtain mixing on the molecular level. This procedure also
20 makes it possible to adjust the pH-value to a desired level, for instance to improve absorption of the polypeptide. The pharmaceutically accepted limits of pH 3.0 to 8.5 for inhalation products must be taken into account, since products with a pH outside these limits may induce irritation and constriction of the airways. To obtain a powder, the solvent must be removed by a process which retains the
25 polypeptide's biological activity. Suitable drying methods include vacuum concentration, open drying, spray drying, and freeze drying. Temperatures over 40°C for more than a few minutes should generally be avoided, as some degradation of certain polypeptides may occur. Following the drying step, the solid material can, if necessary, be ground to obtain a coarse powder, then, if
30 necessary, micronized.

If desired, the micronized powder can be processed to improve the flow properties, e.g., by dry granulation to form spherical agglomerates with superior handling characteristics, before it is incorporated into the intended inhaler device. In such a case, the device would be configured to ensure that the

5 agglomerates are substantially deagglomerated prior to exiting the device, so that the particles entering the respiratory tract of the patient are largely within the desired size range. Where an ordered mixture is desired, the active compound may be processed, for example by micronisation, in order to obtain, if desired, particles within a particular size range. The carrier may also be processed, for example to

10 obtain a desired size and desirable surface properties, such as a particular surface to weight ratio, or a certain ruggedness, and to ensure optimal adhesion forces in the ordered mixture. Such physical requirements of an ordered mixture are well known, as are the various means of obtaining an ordered mixture which fulfills the said requirements, and may be determined easily by the skilled person according to

15 the particular circumstances.

A preferred inhalation apparatus would have the following design characteristics: protection of the powder from moisture and no risk of occasional large doses; in addition as many as possible of the following are desired: protection of the powder from light; high respirable fraction and high lung

20 deposition in a broad flow rate interval; low deviation of dose and respirable fraction; low retention of powder in the mouthpiece - this is particularly important for a multidose inhaler, where polypeptide retained in the mouthpiece could degrade and then be inhaled together with subsequent doses; low adsorption to the inhaler surfaces; flexibility in dose size; and low inhalation resistance. The inhaler

25 is preferably a single dose inhaler although a multi dose inhaler, such as a multi dose, breath actuated, dry powder inhaler for multiple use, may also be employed. Preferably the inhaler used is a unit dose, breath actuated, dry powder inhaler for single use.

A number of dry powder formulations containing a polypeptide and

30 various enhancers have been prepared and tested in an *in vivo* assay, and are

described below. Also described is an *in vitro* assay useful for testing polypeptide/enhancer combinations.

5 Example 1: In vitro method of determining usefulness of particular polypeptides for the present invention.

 A standard *in vitro* assay utilizing an epithelial cell line, CaCo-2 (available through the American Type Culture Collection (ATCC), Rockville, MD, USA), has been developed to assess the ability of various enhancer compounds to promote transport of markers across an epithelial cell monolayer, as a model for
10 the epithelial cell layer which functions in the lung to separate the alveolus from the pulmonary blood supply.

 In this assay, the enhancer and polypeptide or other marker are dissolved in aqueous solution at various proportions and/or concentrations, and applied to the apical side of the cell monolayer. After 60 min incubation at 37°C
15 and 95% RH (relative humidity), the amount of the marker on the basolateral side of the cells is determined, e.g., by use of a radioactively labelled marker.

 For the enhancer tested, sodium caprate, the amount of marker (mannitol, MW 360) which appears on the basolateral side is dependent upon the concentration of enhancer used, at least up to 16 mM sodium caprate (Fig. 1).
20 This is true even when the polypeptide insulin is added to the enhancer/mannitol mixture (1:3 sodium caprate:insulin, by weight) (Fig. 2). This concentration of sodium caprate (16 mM) was also found to promote absorption across the cell monolayer of two low molecular weight peptides, insulin (MW 5734) and vasopressin (MW 1208). The amount of insulin which passed across the
25 monolayer doubled in the presence of 16 mM sodium caprate, compared to the amount in the absence of any enhancer; the amount of vasopressin which was absorbed across the monolayer increased 10-15 times compared to the amount in the absence of any enhancer.

 In contrast, no increase in transport rate was observed for larger
30 proteins such as cytochrome C (MW 12,300), carbonic anhydrase (MW 30,000)

and albumin (MW 69,000) when tested at up to 16 mM sodium caprate. It is expected that at higher concentrations of sodium caprate, the permeability of the cells will be further increased, permitting the transport of larger polypeptides; however, the potential cytotoxicity of sodium caprate may prevent the use of substantially higher concentrations of this particular enhancer.

Other enhancers may permit transportation of larger polypeptides; these may also be tested in this *in vitro* model of epithelial cell permeability, which can be used as a screening tool for rapidly testing any desired polypeptide/enhancer combination for usefulness in the methods of the invention.

10

Example 2: Method for selecting enhancers useful for the present invention.

Each of the compounds listed in Table I was tested for its ability to enhance uptake of a polypeptide (insulin) in a rat model. The results with insulin are taken as indicative of the enhancer's potential for enhancement of absorption of other polypeptides.

15

Various forms of insulin were employed in the different trials: recombinant human, semisynthetic human or bovine. Each formulation was prepared as above, drying and processing the insulin/enhancer or insulin/enhancer/lactose solution to produce an inhalable powder. The powder was administered to rats by inhalation, and the blood glucose levels of the rats were subsequently monitored as a measure of insulin uptake. These levels were compared to the corresponding values obtained from rats which had inhaled insulin formulations without enhancer.

20

The same *in vivo* model system could be used to test any given peptide or protein for usefulness in the methods of the invention, by delivering by the same inhalation method a formulation containing the desired peptide or protein combined with an enhancer, and assaying for the concentration of the desired peptide or protein in the systemic circulation of the test animal (e.g., by standard immunoassays or biochemical assays as appropriate for the given peptide or

25

30

protein).

TABLE I

| | <u>Substance</u> | <u>Enhancer:Insulin:lactose</u> | <u>Effect</u> |
|----|---------------------------------|---------------------------------|---------------|
| | Octylglucopyranoside | 4:4:92 | (+) |
| 5 | Sodium ursodeoxycholate | 4:4:92 | + |
| | Sodium taurocholate | 4:4:92 | + |
| | Sodium glycocholate | 4:4:92 | + |
| | Lysophosphatidylcholine | 4:4:92 | + |
| | Diocanoylphosphatidylcholine | 2:4:94 | (+) |
| 10 | Didecanoylphosphatidylcholine | 4:4:94 | - |
| | Sodium taurodihydrofusidate | 2:4:94 | + |
| | Sodium caprylate | 25:75:0 | + |
| | Sodium caprate | 10:90:0 | (+) |
| | Sodium caprate | 17.5:82.5:0 | (+) |
| 15 | Sodium caprate | 25:75:0 | + |
| | Sodium caprate | 4:4:92 | + |
| | Sodium laurate | 25:75:0 | (+) |
| | Potassium oleate | 4:4:92 | + |
| | Potassium caprate | 27:73:0 | + |
| 20 | Lysine caprate | 35:65:0 | + |
| | Sodium myristate | 30:70:0 | + |
| | Dimethyl- β -cyclodextrin | 75:25:0 | + |

- + effect, i.e. enhancer gives a significant decrease in blood glucose level
 25 - no or very small effect
 (+) effect, not as marked as "+"

Example 3: Therapeutic preparation according to the invention.

Human growth hormone (hGH, MW 22kD, source Humatrope from
 30 Lilly, 3 parts) was mixed with sodium caprate (1 part). The mixture was milled in

a Retsch mechanical mill to a particle size of mass median diameter 6.7 μm .

The resultant powder was administered intratracheally in rats and the uptake of hGH compared with that of a powder, MMD 9.6 μm , comprising hGH and mannitol in the same proportions and prepared in the same way as above.

5 The results indicated an improvement in the uptake of hGH in the formulation including sodium caprate, compared with the uptake in the formulation without enhancer.

10 Example 4: Preparation containing the polypeptide insulin.

Insulin is herein used as indicative of other polypeptides according to the present invention.

15 Biosynthetic human insulin (53g) was micronised in an Airfilco Jet Mill (Trade Mark, Airfilco Process Plant Limited), with pressurised nitrogen (feed pressure 7 bar, chamber pressure 5 bar), to a mass median diameter of 2.4 micrometers.

Sodium caprate (170g) was micronised in an Airfilco Jet Mill (TM), with pressurised nitrogen (feed pressure 5 bar, chamber pressure 3 bar), to a mass median diameter of 1.6 micrometers.

20 The micronised biosynthetic human insulin (45g) and sodium caprate (14.26g) were dry mixed according to the following procedure: Half of the insulin was added to a mixing device comprising a mixing cylinder of volume 4.4 litres divided, by a sieve of width 1mm, into two compartments, with a metal ring in each compartment to aid mixing and stirring. The sodium caprate and finally the
25 rest of the insulin, were added. The mixing cylinder was closed, turned 180 degrees, and mounted in a motorised shaking apparatus. The motor was turned on and shaking continued for approximately two minutes, until all the insulin and sodium caprate had passed through the sieve. The motor was turned off and the mixing cylinder turned 180 degrees, again mounted on the shaking apparatus and
30 shaking was again effected until all the powder had passed through the sieve. This

procedure was repeated a further eight times to give a total mixing time of approximately 20 minutes.

The preparation so obtained was administered to 5 dogs by inhalation, at a dosage level of 1 U./kg, and the plasma insulin level determined at various time points after administration.

The results obtained were compared with the plasma insulin levels obtained when biosynthetic insulin, micronised as above to a mass median diameter of 2.4 micrometers, were administered to five dogs in the same way and at the same dosage levels, and with the plasma insulin levels obtained when a therapeutic preparation of insulin and sodium caprate in a ratio of 90:10 was administered to five dogs in the same way and at the same dosage levels as above. In this case the therapeutic preparation was prepared as follows: Human semisynthetic insulin was gel filtrated to reduce the zinc content from 0.52% to 0.01% relative to content of insulin. Insulin (4.5g) and sodium caprate (0.5g) were dissolved in water (232 ml). The solution was stirred until clear and the pH adjusted to 7.0. The solution was concentrated by evaporation at 37°C over a period of about two days. The obtained solid cake was crushed, and sieved through a 0.5 mm sieve, and the resultant powder micronised through a jet mill to particles with a mass median diameter of 3.1 micrometers.

The results of these comparisons are presented in Figure 3 ($p=0.0147$ for the difference between 75:25 and 100:0). The results demonstrate some improvement in the bioavailability of insulin with the 90:10 formulation, and a dramatic improvement in the bioavailability of insulin with the 75:25 preparation including sodium caprate, as compared to insulin alone.

What is claimed is:

1. A pharmaceutical composition, comprising a mixture of active compounds (A) a pharmaceutically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of said polypeptide in the lower respiratory tract of a patient, said mixture being in the form of a dry powder for inhalation, in which at least 50% of the total mass of active compounds consists of primary particles having a diameter less than or equal to about 10 microns, said primary particles optionally being formed into agglomerates.
2. A pharmaceutical composition as claimed in claim 1, additionally comprising a pharmaceutically acceptable carrier, which comprises either
 - (a) particles having a diameter of less than about 10 microns, such that at least 50 % of the resultant powder consists of optionally agglomerated primary particles having a diameter of less than about 10 microns; or
 - (b) coarse particles, such that an ordered mixture is formed between the active compounds and the said carrier.
3. The composition of claim 1, wherein said polypeptide is a polypeptide hormone.
4. The composition of claim 3, wherein said hormone is vasopressin, a vasopressin analogue, desmopressin, glucagon, corticotropin (ACTH), gonadotrophin (luteinizing hormone, or LHRH), calcitonin, C-peptide of insulin, parathyroid hormone (PTH), human growth hormone (hGH), growth hormone (HG), growth hormone releasing hormone (GHRH), oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs, gonadotropin agonist analogs (GnRHa), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRHrh), follicle stimulating hormone (FSH), or prolactin.

5. The composition of claim 1, wherein said polypeptide is a growth factor, interleukin, polypeptide vaccine, enzyme, endorphin, glycoprotein, lipoprotein, or polypeptide involved in the blood coagulation cascade, that exerts its pharmacological effect systemically.

5

6. The composition of claim 1, wherein said polypeptide has a molecular weight of less than 30 kD.

7. The composition of claim 1, wherein said polypeptide has a
10 molecular weight of less than 25 kD.

8. The composition of claim 1, wherein said polypeptide has a molecular weight of less than 20 kD.

9. The composition of claim 1, wherein said polypeptide has a
15 molecular weight of less than 15 kD.

10. The composition of claim 1, wherein said polypeptide has a
20 molecular weight of less than 10 kD.

11. The composition of claim 1, wherein said enhancer compound is a surfactant.

12. The composition of claim 11, wherein said surfactant is a bile
25 salt, a bile salt derivative, an alkyl glycoside, a cyclodextrin or derivative thereof, or a phospholipid.

13. The composition of claim 11, wherein said surfactant is a salt of
30 a fatty acid.

14. The composition of claim 11, wherein said fatty acid has 10-14 carbon atoms.

5 15. The composition of claim 14, wherein said fatty acid is capric acid.

16. The composition of claim 11, wherein said surfactant is sodium caprate.

10 17. An inhaler device containing the composition of claim 1.

18. The inhaler device of claim 17, wherein said composition is in the form of said agglomerates, said device being configured to induce the majority of said agglomerates to break down into particles having a diameter less than or
15 equal to about 10 microns, upon inhalation of said agglomerates from said device.

19. The inhaler device of claim 17, which inhaler device is a unit dose, breath actuated, dry powder inhaler for single use.

20 20. The inhaler device of claim 17, which inhaler device is a multi dose, breath actuated, dry powder inhaler for multiple use.

21. A method for systemic administration of a pharmaceutically active polypeptide, comprising
25 providing a composition comprising a mixture of active compounds (A) a pharmaceutically active polypeptide and (B) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory tract of a patient, said composition being in the form of a dry powder; and
causing said patient to inhale said composition; provided that the
30

diameter of the particles of the active compounds at the point they enter the respiratory tract of the patient is less than or equal to about 10 microns.

22. The method of claim 21, wherein said composition is inhaled from an inhaler device which contains said powder in the form of agglomerates of said particles, said agglomerates being substantially deagglomerated prior to entering the respiratory tract of said patient.

23. A process for the manufacture of a pharmaceutical composition suitable for administration by inhalation, comprising

providing a solution in which are dissolved (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the systemic absorption of the polypeptide in the lower respiratory tract of a patient;

removing the solvent from said solution to yield a dry solid comprising said polypeptide and said enhancer compound; and pulverizing said dry solid to produce a powder.

24. A process for the preparation of a pharmaceutical composition suitable for administration by inhalation, comprising

dry mixing (a) a pharmaceutically active polypeptide and (b) an enhancer compound which enhances the absorption of the polypeptide in the lower respiratory tract of a patient; and

micronizing the obtained mixture.

25. A process for the manufacture of a pharmaceutical composition suitable for administration by inhalation, comprising

providing a first micronized preparation comprising a polypeptide and a second micronized preparation comprising an enhancer compound which enhances the absorption of the polypeptide in the lung of a patient; and mixing said first and second micronized preparations.

26. Use of an enhancer in the preparation of an inhalable dry powder preparation of a polypeptide or analogue or modified or truncated derivative thereof, with enhanced systemic absorption of said polypeptide in the lower respiratory tract, in which at least 50% of total mass of polypeptide and enhancer consists of (1) particles having a diameter of 10 microns or less, or (2) agglomerates of said particles.

27. Use according to claim 26, wherein the polypeptide is a polypeptide hormone.

28. Use according to claim 27, wherein said hormone is vasopressin, a vasopressin analogue, desmopressin, glucagon, corticotropin (ACTH), gonadotrophin (luteinizing hormone, or LHRH), calcitonin, C-peptide of insulin, parathyroid hormone (PTH), human growth hormone (hGH), growth hormone (HG), growth hormone releasing hormone (GHRH), oxytocin, corticotropin releasing hormone (CRH), somatostatin analogs, gonadotropin agonist analogs (GnRH_a), atrial natriuretic peptide (hANP), thyroxine releasing hormone (TRH_{rh}), follicle stimulating hormone (FSH), or prolactin.

29. Use according to claim 26, wherein the enhancer is a surfactant.

30. Use according to claim 29, wherein the enhancer is a salt of a fatty acid.

31. Use according to claim 30, wherein the enhancer is sodium caprate.

- 1 / 2 -

Fig. 1

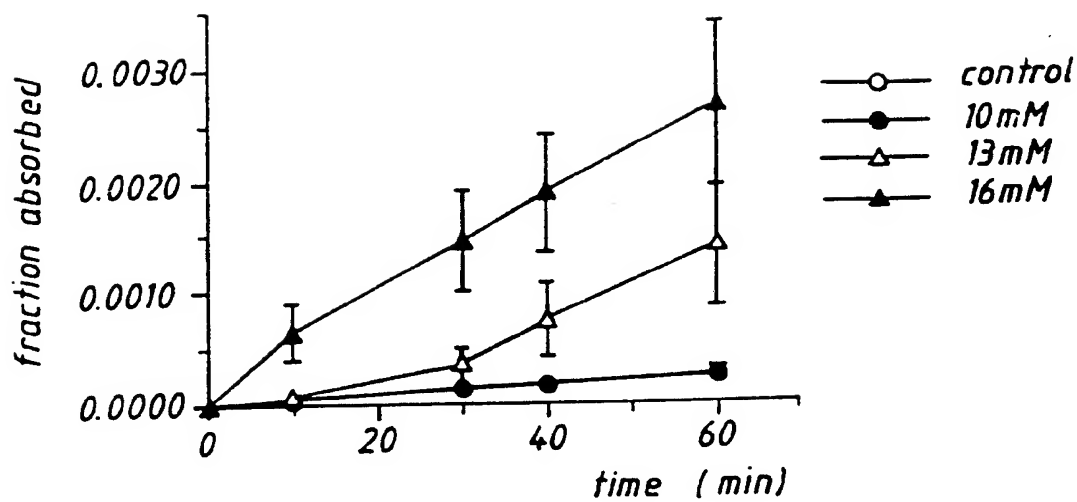
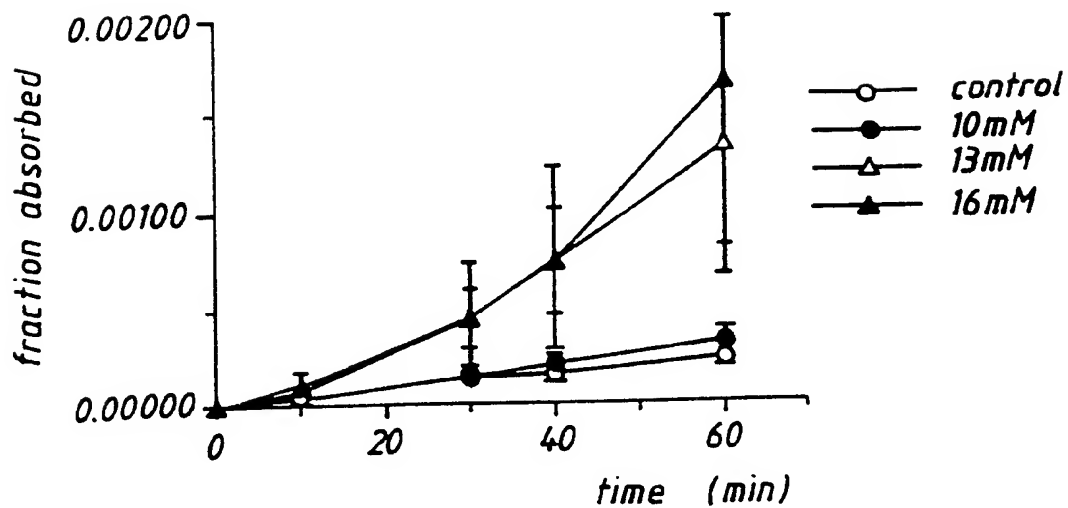
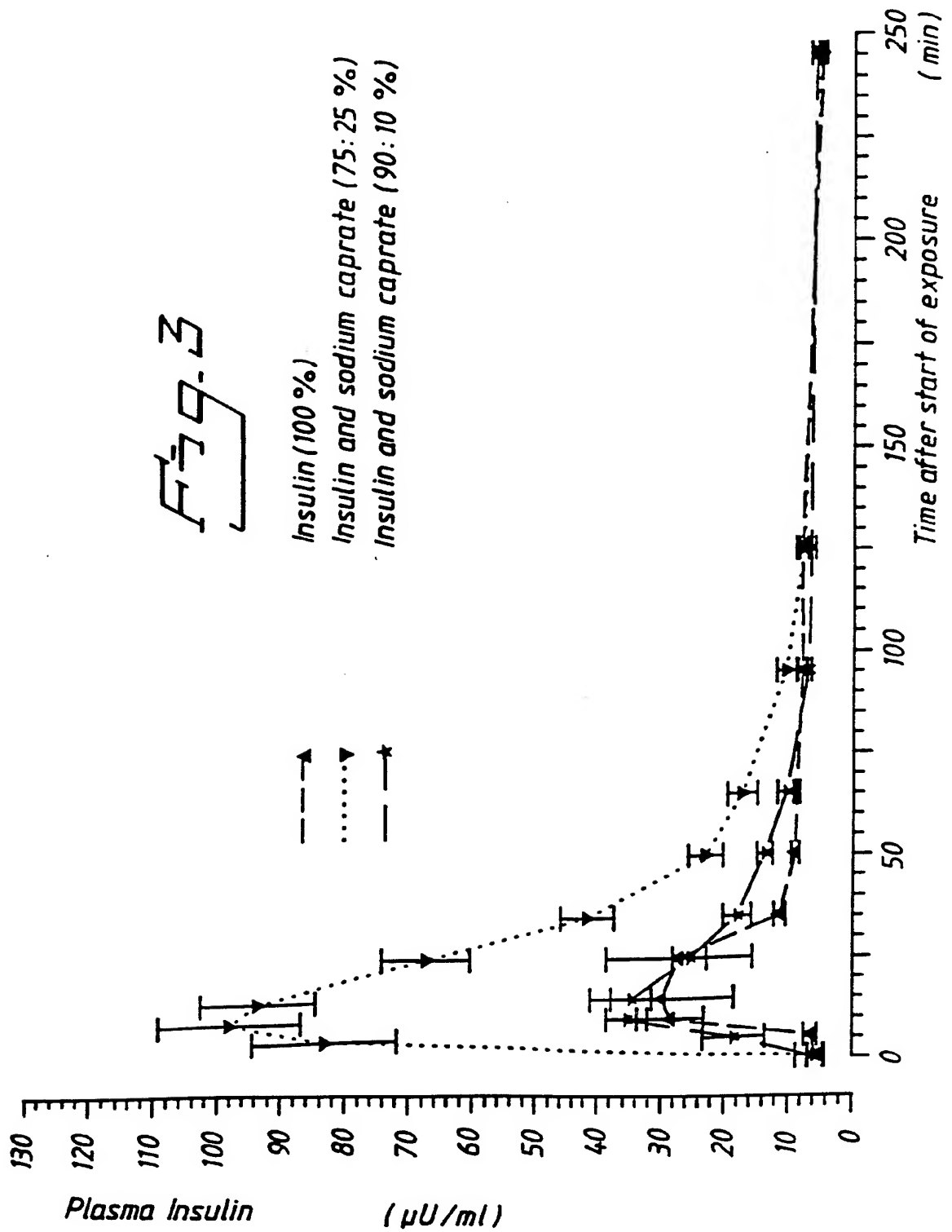


Fig. 2



SUBSTITUTE SHEET

- 2 / 2 -



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00634

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 9/72, A61K 9/14, A61K 37/26, A61K 47/12
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EMBASE, MEDLINE, CA, WPI, WPIL, CLAIMS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|--|-----------------------|
| X | WO, A1, 9116038 (TORAY INDUSTRIES, INC.), 31 October 1991 (31.10.91) -- | 1,2A,5-8, 13-16,22 |
| X | WO, A1, 8809163 (COSMAS-DAMIAN LIMITED), 1 December 1988 (01.12.88) -- | 1-20,23-31 |
| A | WO, A1, 9004962 (KABIVITRUM AB), 7 May 1990 (07.05.90), page 6, line 3 - page 8, line 6 -- | 2B |

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

22 November 1994

Date of mailing of the international search report

25-11-1994

Name and mailing address of the ISA/
Swedish Patent Office
Box 5055, S-102 42 STOCKHOLM
Facsimile No. +46 8 666 02 86

Authorized officer

Anneli Jönsson

Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00634

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| A | <p>Chemical Abstracts, Volume 108, No 6, 8 February 1988 (08.02.88), (Columbus, Ohio, USA), Mishima, Motohiro et al, "Studies on the promoting effects of medium chain fatty acid salts on the nasal absorption of insulin in rats", page 398, THE ABSTRACT No 43961e, J: Pharmacobio-Dyn. 1987, 10 (11), 624-631</p> <p style="text-align: center;">--</p> | 1-20,23-31 |
| A | <p>US, A, 4613500 (YOSHIKI SUZUKI ET AL), 23 Sept 1986 (23.09.86), examples</p> <p style="text-align: center;">-- -----</p> | 23-25 |

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 94/00634

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 21-22
because they relate to subject matter not required to be searched by this Authority, namely:

Methods for treatment of the human or animal body by surgery or therapy,
as well as diagnostic methods (see PCT Rule 39(iv)).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such
an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Group I: Particles with active substances (polypeptide and an enhancer compound) mixed with carrier which comprises particles having a diameter of less than about 10 μm .
Claims 1, 2a, 3-20, 23-31.

Group II: Particles with active substances (polypeptide and an enhancer compound) mixed with coarse particles with the size 20-800 μm , such that an ordered mixture is formed.
Claims 2b

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

29/10/94

International application No.

PCT/SE 94/00634

| Patent document cited in search report | | Publication date | Patent family member(s) | | Publication date |
|---|---------|---------------------|----------------------------|----------|---------------------|
| WO-A1- | 9116038 | 31/10/91 | EP-A- | 0477386 | 01/04/92 |
| | | | JP-A- | 5000963 | 08/01/93 |
| WO-A1- | 8809163 | 01/12/88 | AU-B- | 614290 | 29/08/91 |
| | | | AU-A- | 1793188 | 21/12/88 |
| | | | DE-D,T- | 3888201 | 14/07/94 |
| | | | EP-A,B- | 0391896 | 17/10/90 |
| | | | SE-T3- | 0391896 | |
| | | | GB-A- | 2231495 | 21/11/90 |
| | | | JP-T- | 2503915 | 15/11/90 |
| WO-A1- | 9004962 | 07/05/90 | AU-A- | 4480889 | 28/05/90 |
| | | | DE-D,T- | 68914683 | 27/10/94 |
| | | | EP-A,B- | 0440702 | 14/08/91 |
| | | | SE-T3- | 0440702 | |
| | | | SE-B,C- | 466088 | 16/12/91 |
| | | | SE-A- | 8803935 | 31/10/88 |
| | | | SE-A- | 9101027 | 08/04/91 |
| US-A- | 4613500 | 23/09/86 | EP-A,B,B | 0122036 | 17/10/84 |
| | | | SE-T3- | 0122036 | |
| | | | JP-C- | 1667473 | 29/05/92 |
| | | | JP-A- | 59163313 | 14/09/84 |
| | | | JP-B- | 62037016 | 10/08/87 |